FIGURE 1

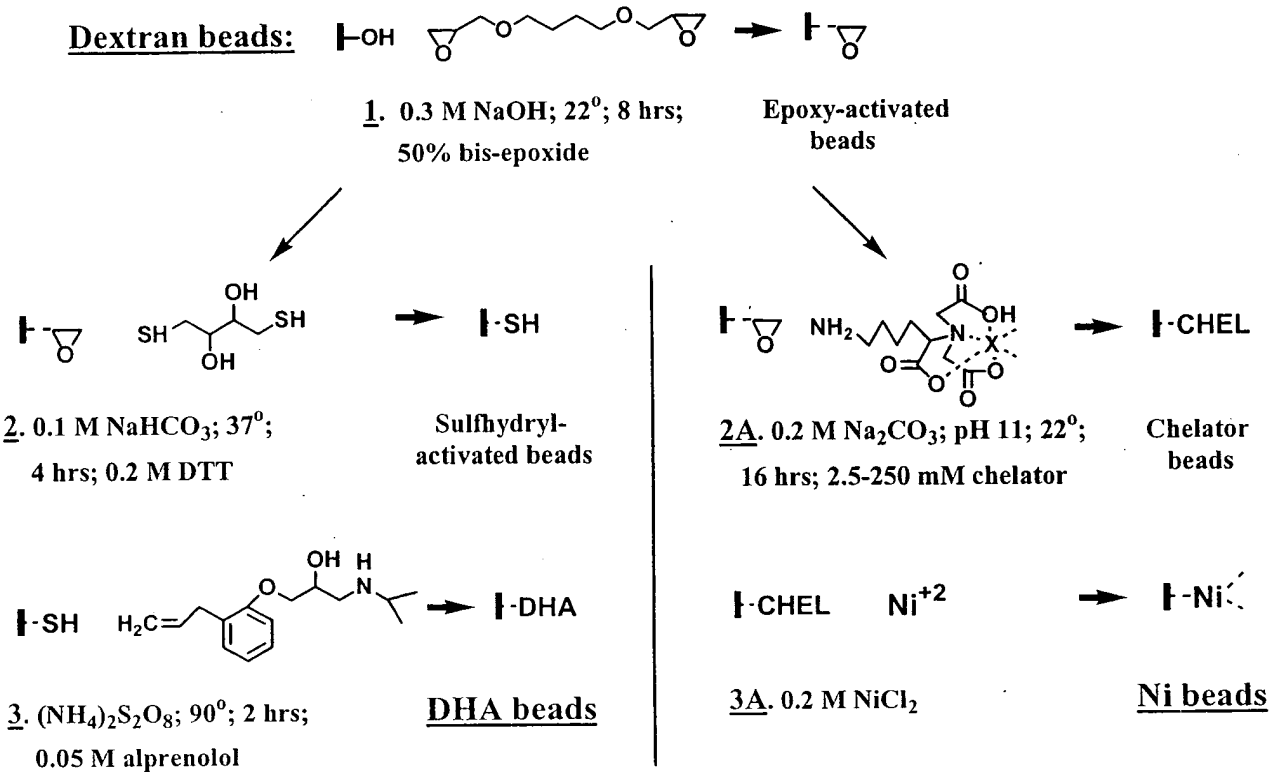

Dextran beads:
1. 0.3 M NaOH; 22°; 8 hrs; 50% bis-epoxide
Epoxy-activated beads
2. 0.1 M NaHCO3; 37°; 4 hrs; 0.2 M DTT
Sulfhydryl-activated beads
2A. 0.2 M Na2CO3; pH 11; 22°; 16 hrs; 2.5-250 mM chelator
Chelator beads
3. (NH4)2S2O8; 90°; 2 hrs; 0.05 M alprenolol
DHA beads
3A. 0.2 M NiCl2
Ni beads

FIGURE 2

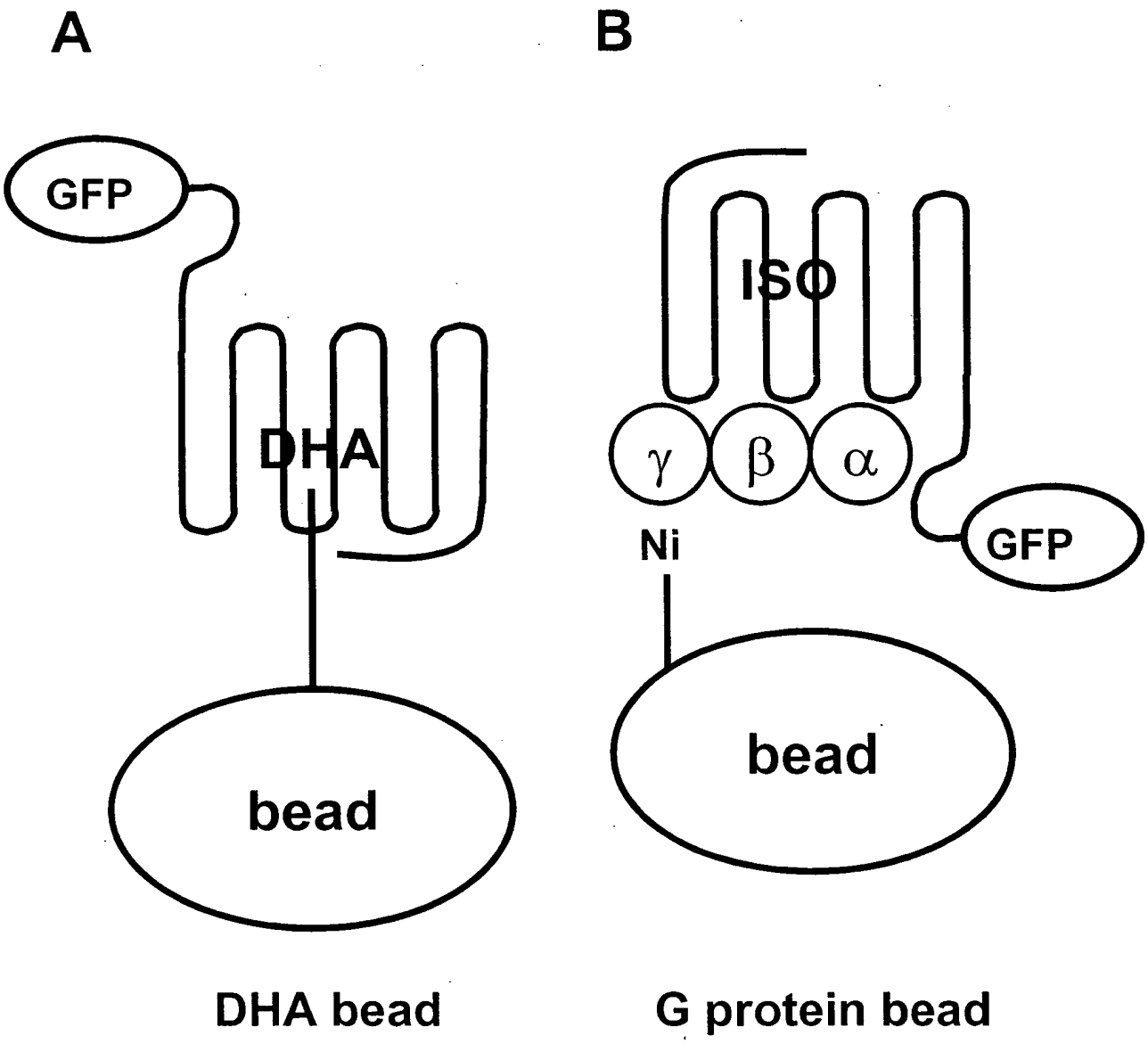


FIGURE 3

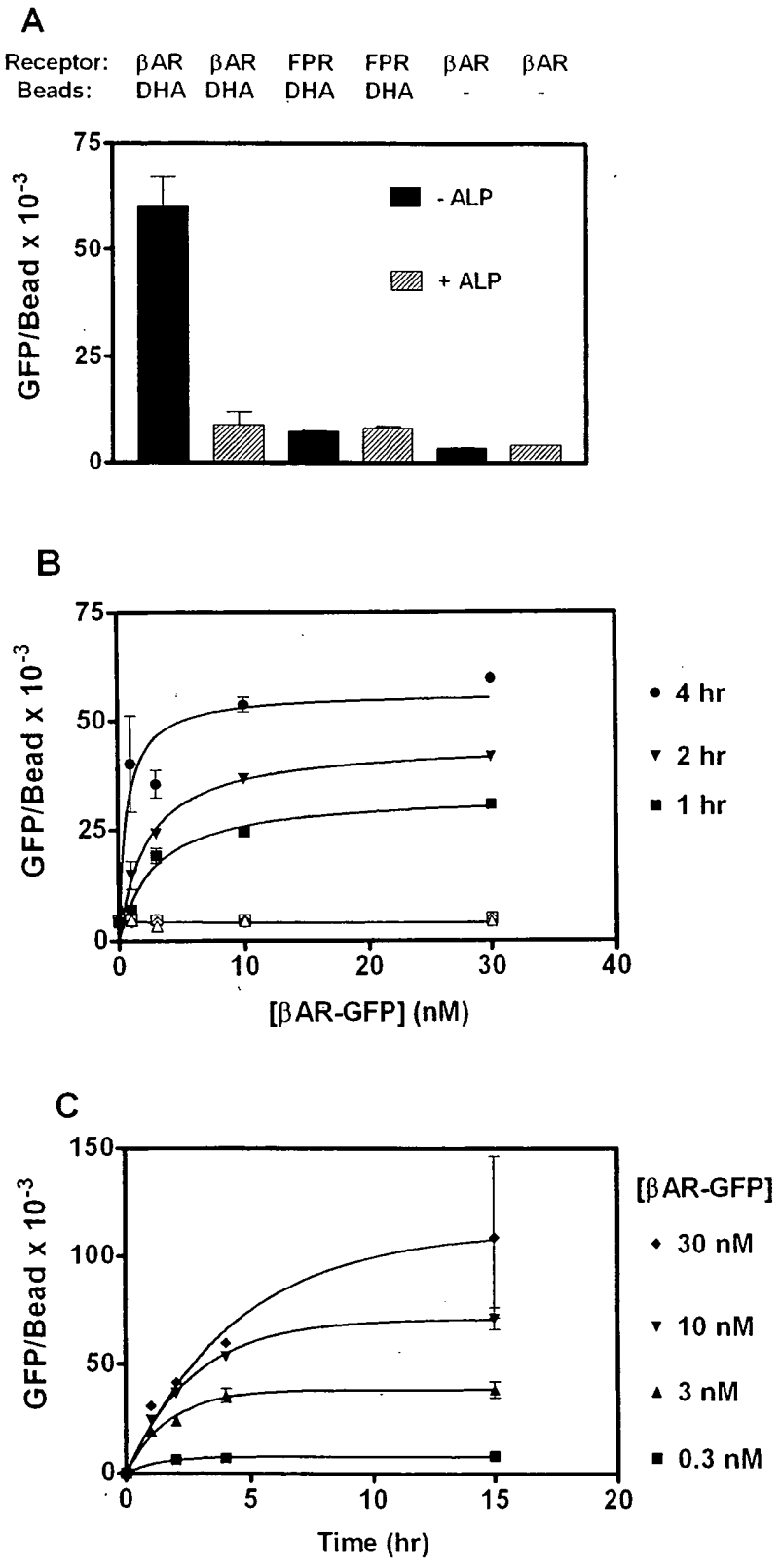

A
Receptor: βAR βAR FPR FPR βAR βAR
Beads: DHA DHA DHA DHA - -
GFP/Bead x 10-3
75
50
25
0
- ALP
+ ALP
B
GFP/Bead x 10-3
75
50
25
0
0
10
20
30
40
[βAR-GFP] (nM)
4 hr
2 hr
1 hr
C
GFP/Bead x 10-3
150
100
50
0
0
5
10
15
20
Time (hr)
[βAR-GFP]
30 nM
10 nM
3 nM
0.3 nM

FIGURE 4

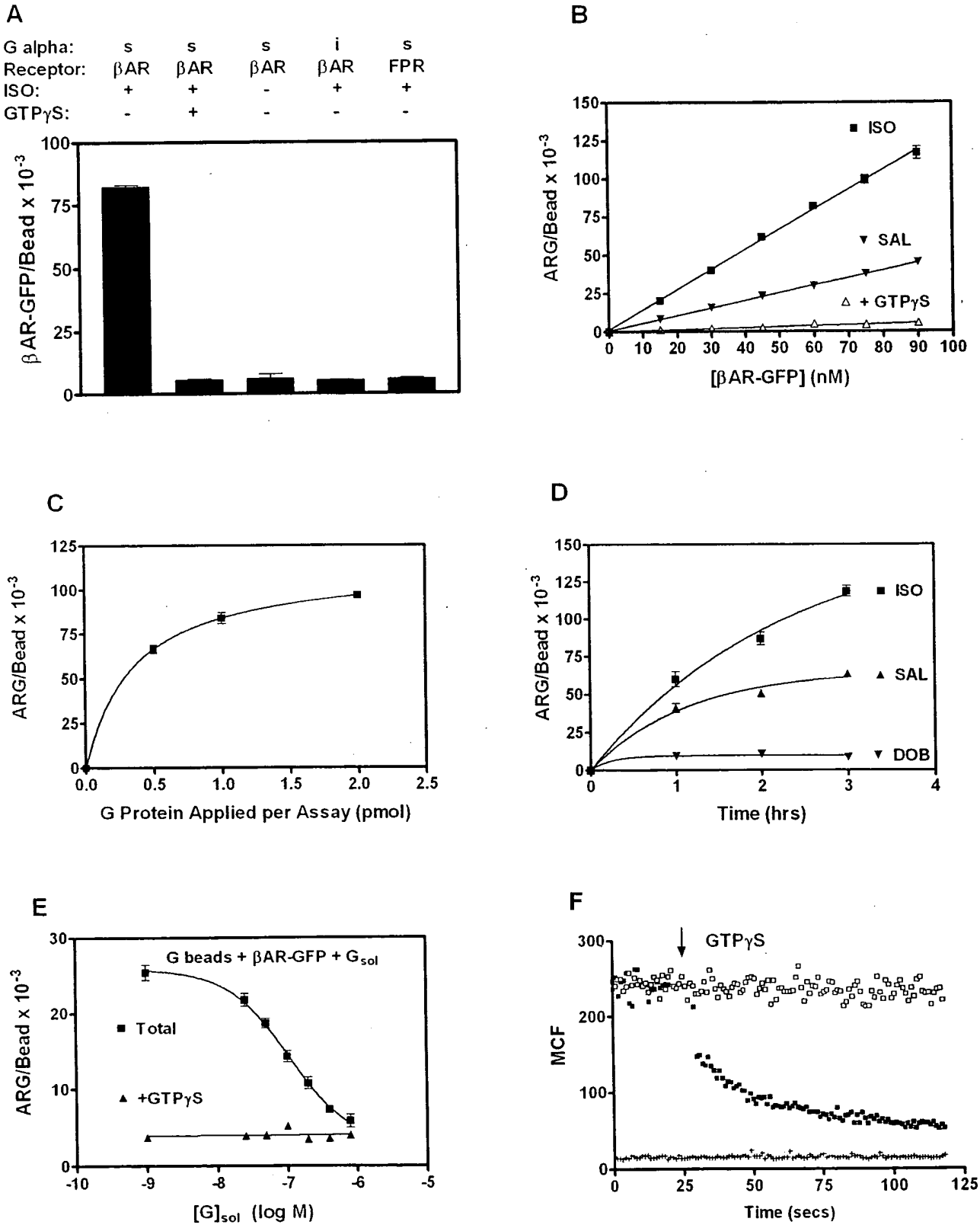
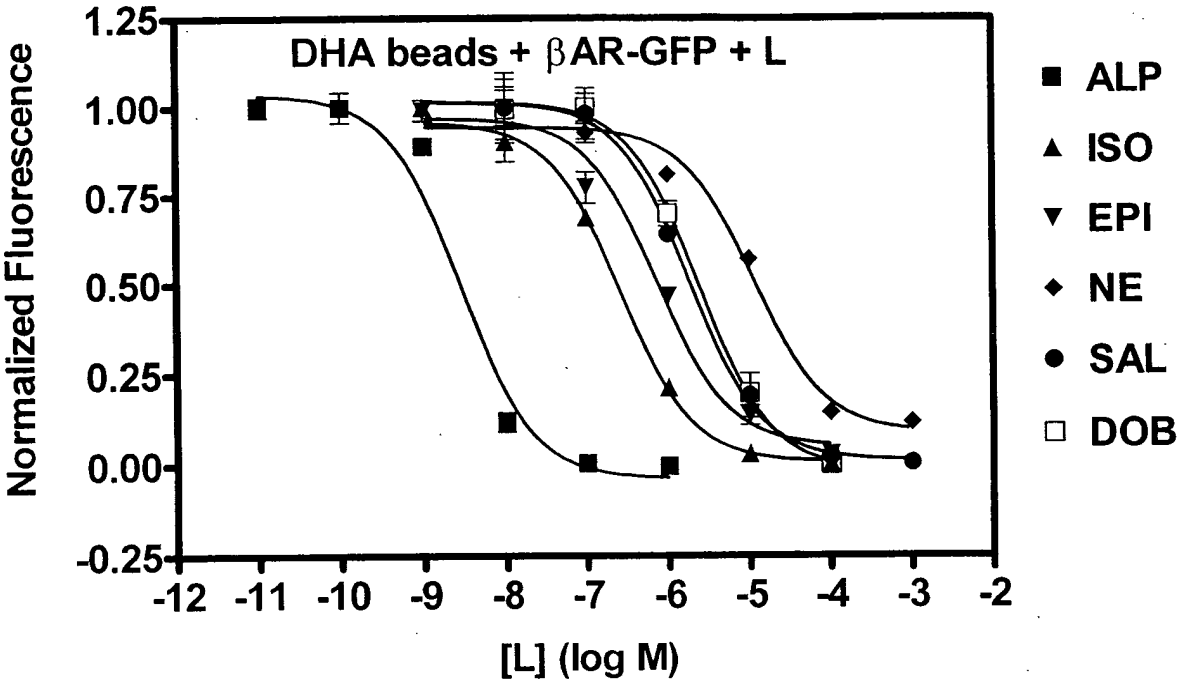

A
G alpha: s s s i s
Receptor: βAR βAR βAR βAR FPR
ISO: + + - + +
GTPγS: - + - - -
βAR-GFP/Bead x 10-3
B
ARG/Bead x 10-3
ISO
SAL
+ GTPγS
[βAR-GFP] (nM)
C
ARG/Bead x 10-3
G Protein Applied per Assay (pmol)
D
ARG/Bead x 10-3
ISO
SAL
DOB
Time (hrs)
E
G beads + βAR-GFP + Gsol
Total
+GTPγS
ARG/Bead x 10-3
[G]sol (log M)
F
GTPγS
MCF
Time (secs)

FIGURE 5

A


DHA beads + βAR-GFP + L
Normalized Fluorescence
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2
[L] (log M)
ALP
ISO
EPI
NE
SAL
DOB

B

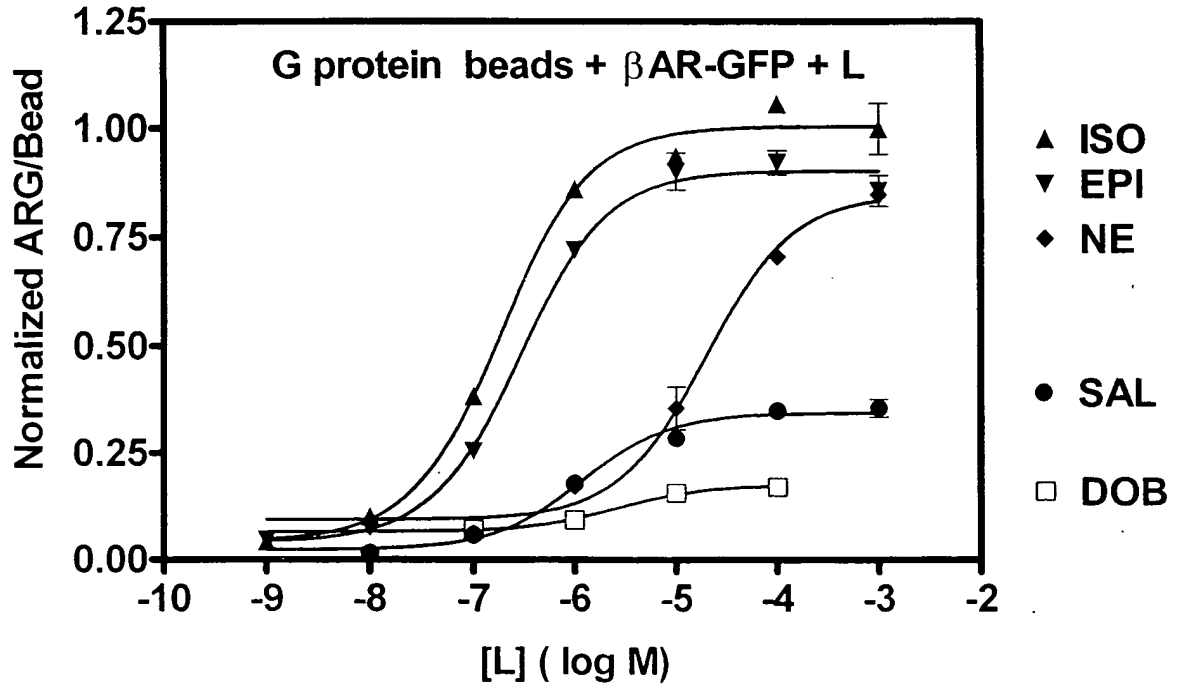
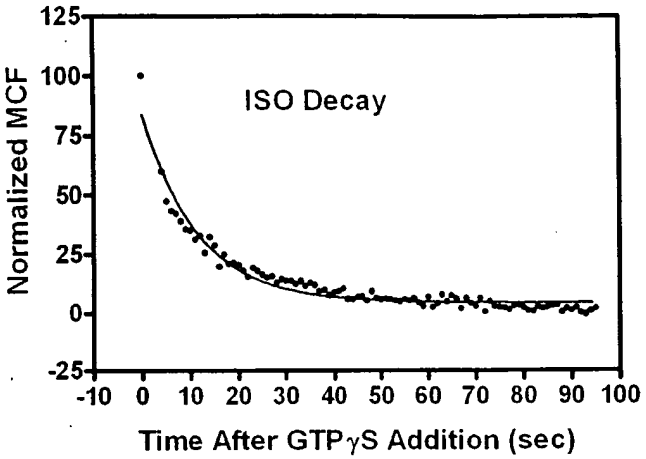
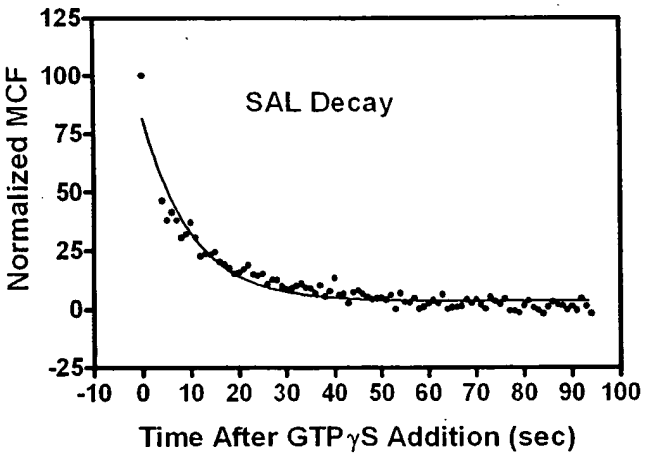

G protein beads + βAR-GFP + L
Normalized ARG/Bead
1.25
1.00
0.75
0.50
0.25
0.00
-10 -9 -8 -7 -6 -5 -4 -3 -2
[L] (log M)
ISO
EPI
NE
SAL
DOB

FIGURE 6


ISO Decay
Normalized MCF
125
100
75
50
25
0
-25
-10 0 10 20 30 40 50 60 70 80 90 100
Time After GTPγS Addition (sec)


SAL Decay
Normalized MCF
125
100
75
50
25
0
-25
-10 0 10 20 30 40 50 60 70 80 90 100
Time After GTPγS Addition (sec)

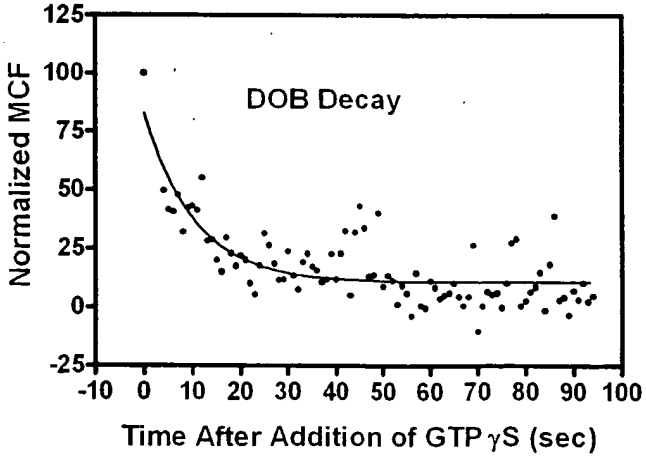

DOB Decay
Normalized MCF
125
100
75
50
25
0
-25
-10 0 10 20 30 40 50 60 70 80 90 100
Time After Addition of GTPγS (sec)

FIGURE 7

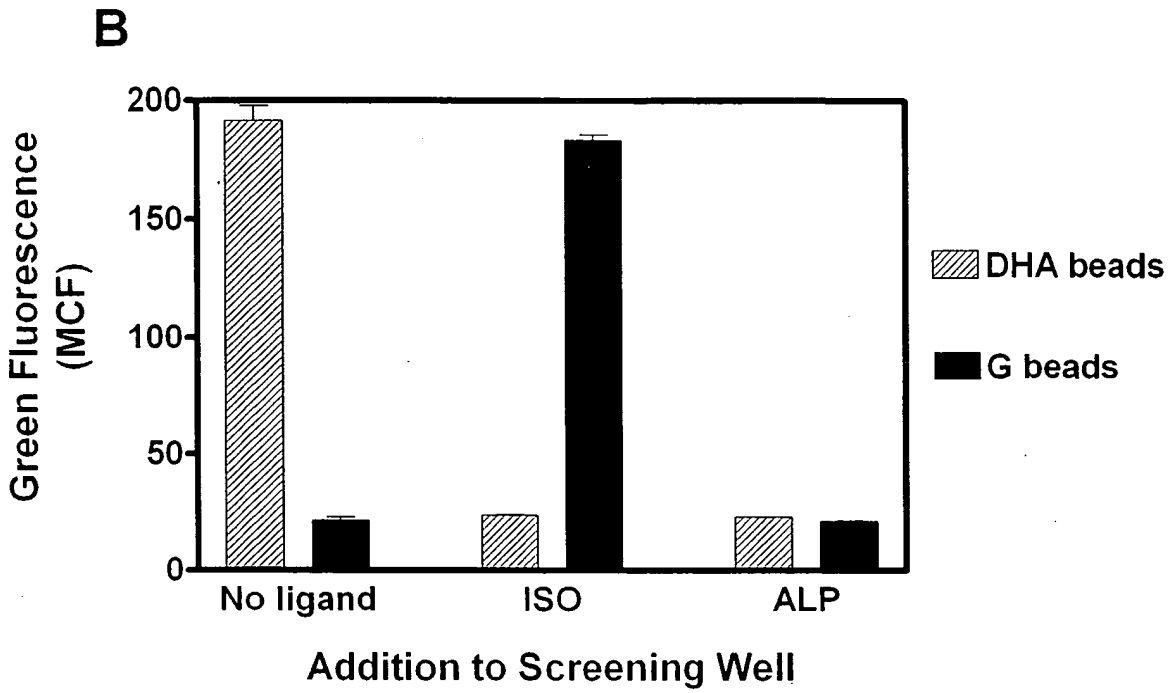

B
200
150
100
50
0
Green Fluorescence (MCF)
No ligand
ISO
ALP
Addition to Screening Well
DHA beads
G beads

FIGURE 8A

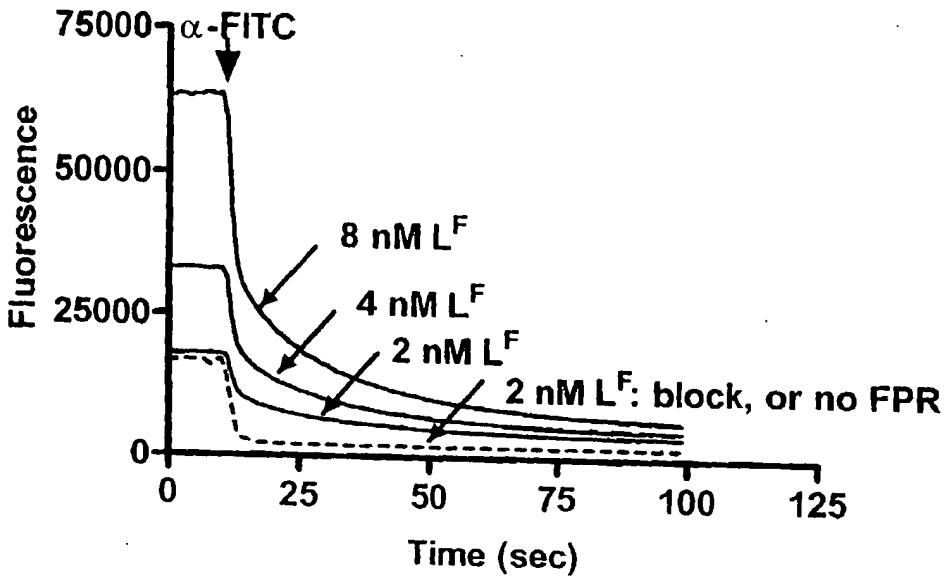


FIGURE 8B

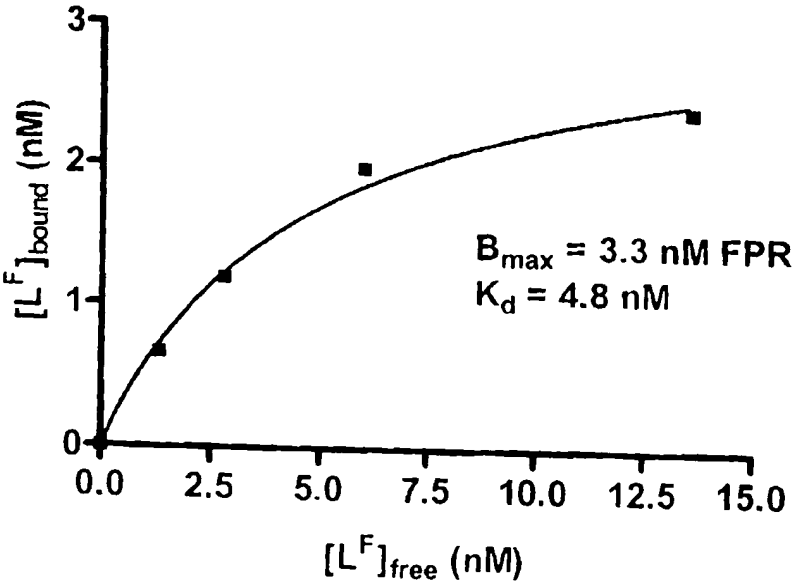


FIGURE 9A

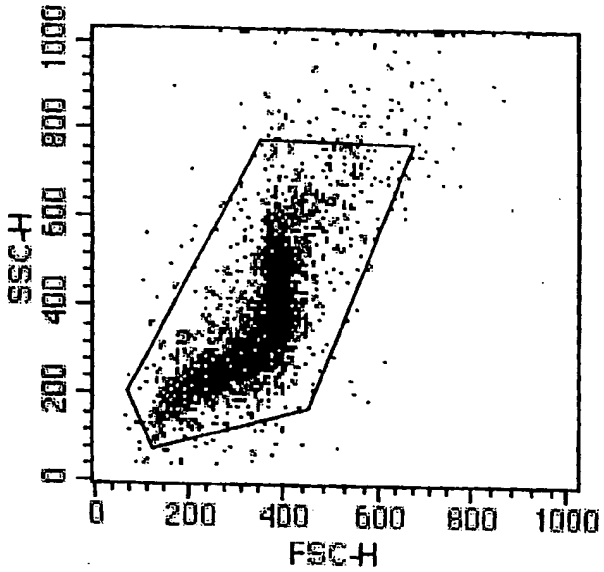


FIGURE 9B

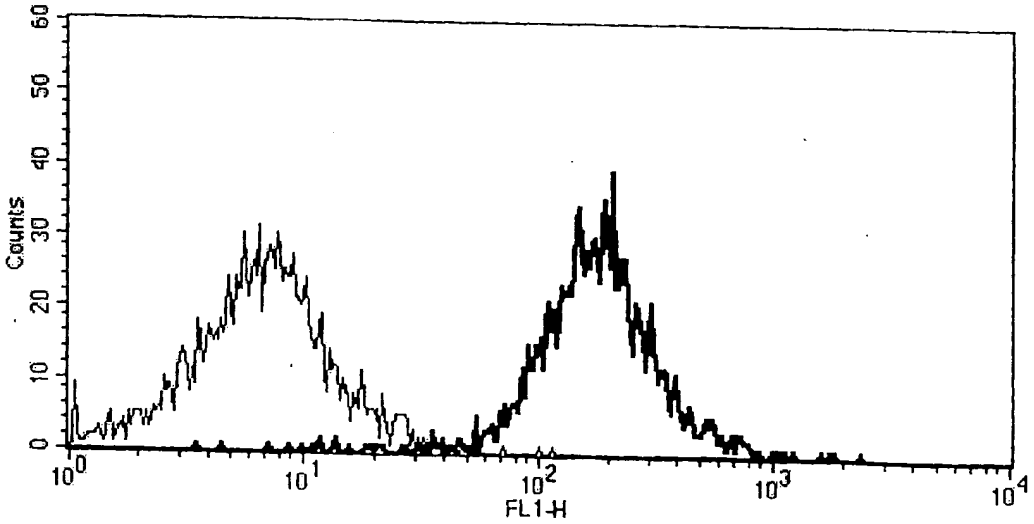


FIGURE 9C

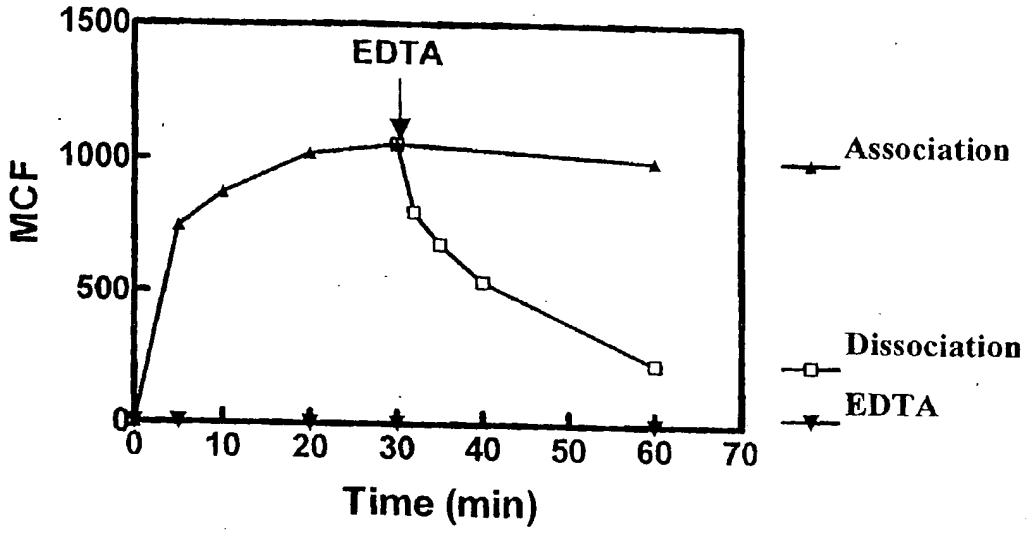


FIGURE 9D

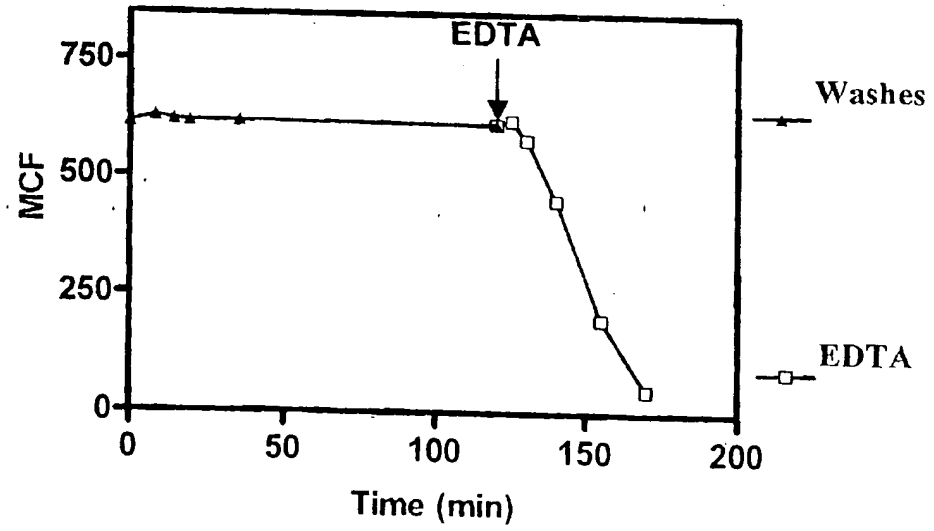


FIGURE 9E

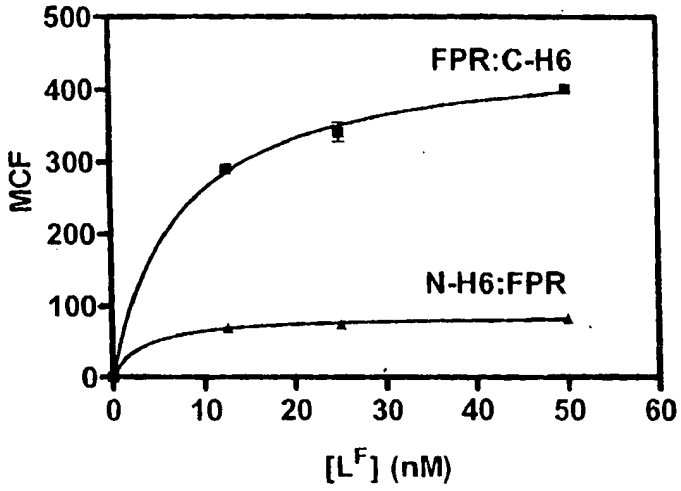


FIGURE 9F

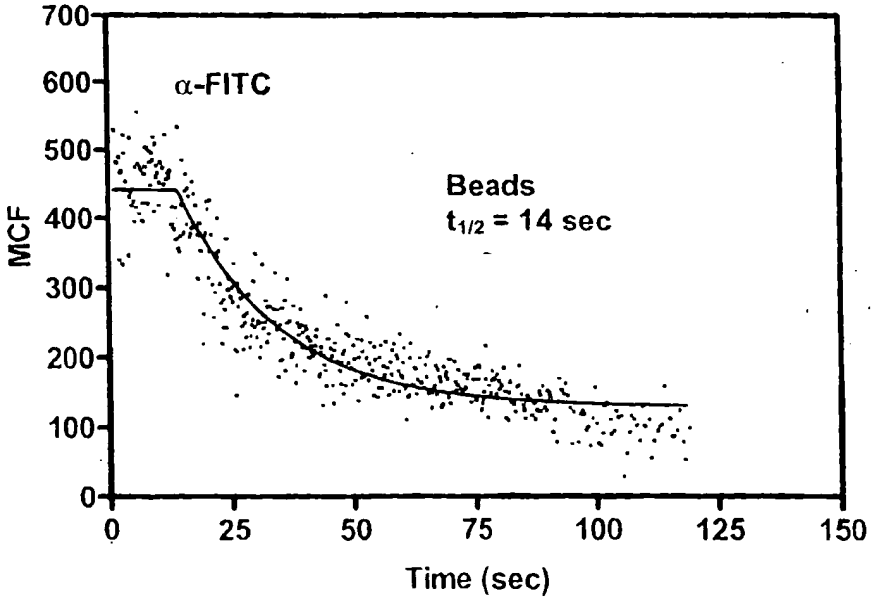


FIGURE 10

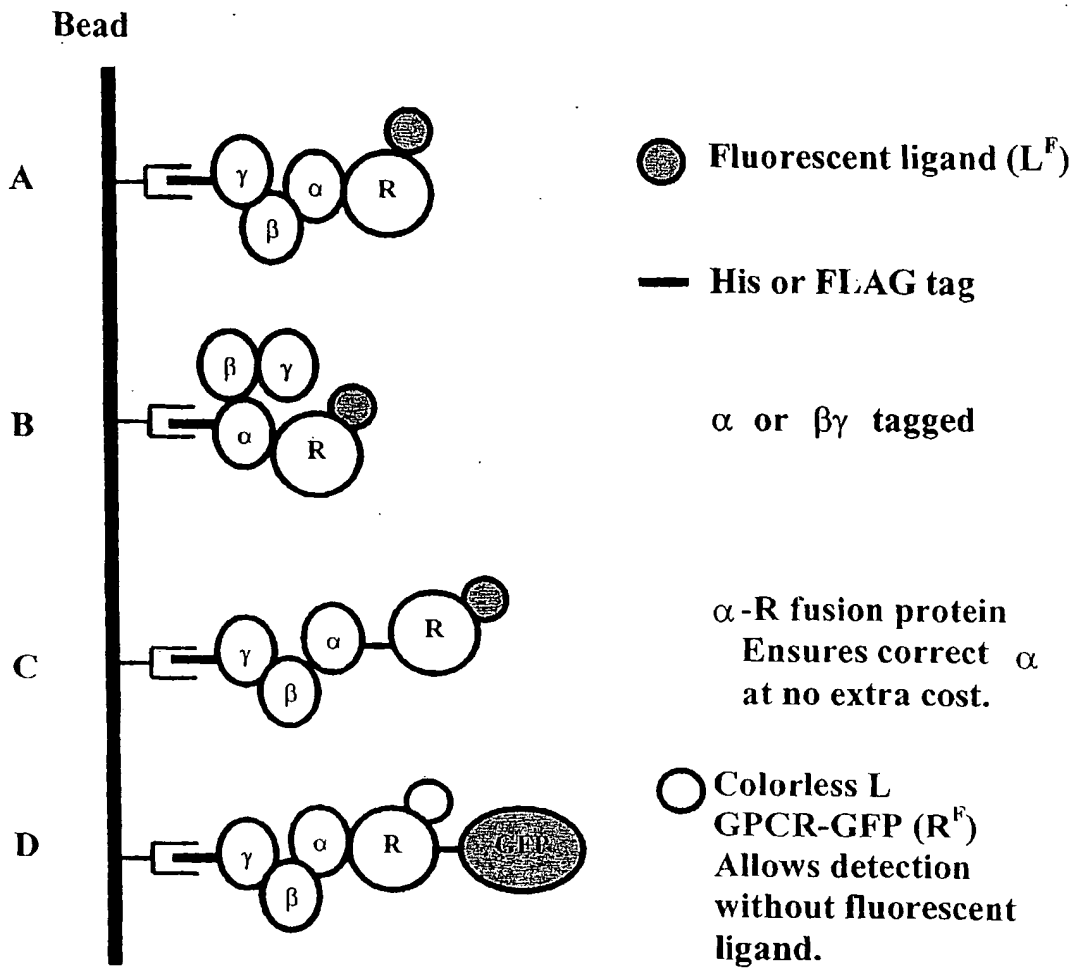
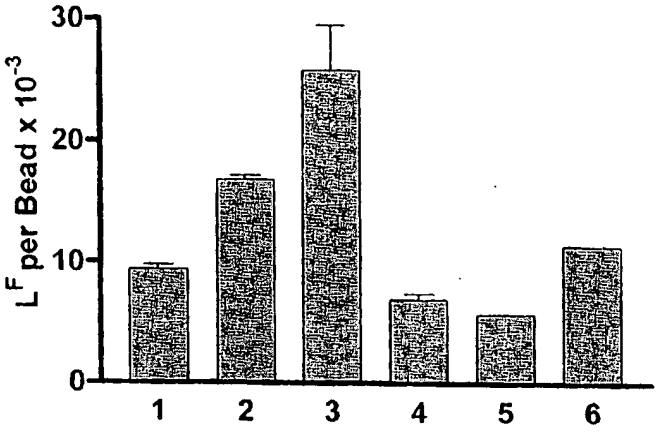

Bead
A
B
C
D
γ
α
β
R
GFP
Fluorescent ligand (L^F)
His or FLAG tag
α or βγ tagged
α-R fusion protein
Ensures correct α
at no extra cost.
Colorless L
GPCR-GFP (R^F)
Allows detection
without fluorescent
ligand.

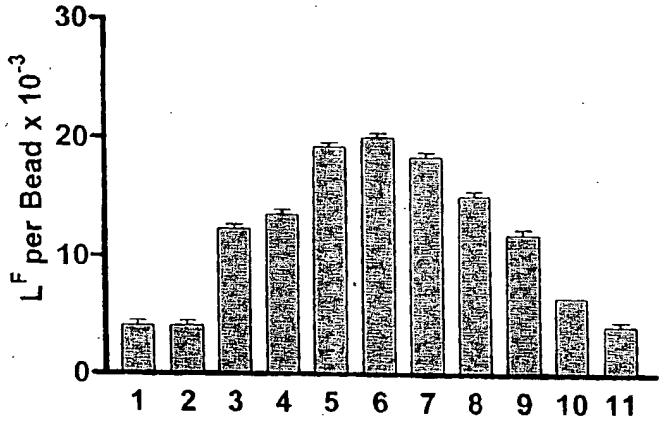
FIGURE 11A



Changes from the standard assembly:

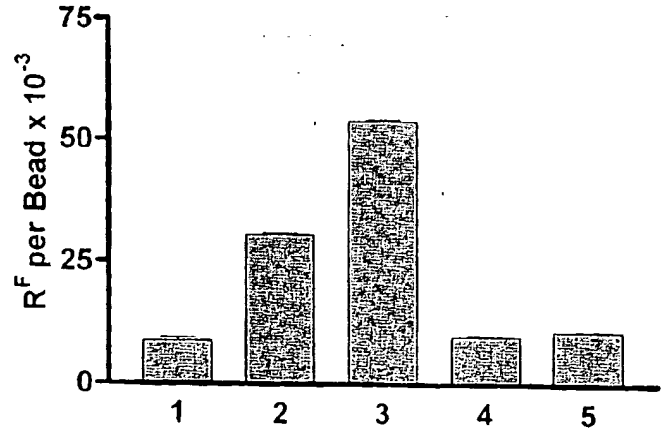
1. No αβγ
2. No α
3. (standard)
4. +GTPγS
5. Irrelevant L-fl
6. No R

FIGURE 11B



1. No βγ
2. +GTPγS
3. (standard)
4. 0.1 μM GTP
5. 0.5 μM GTP
6. 1 μM GTP
7. 5 μM GTP
8. 10 μM GTP
9. 25 μM GTP
10. 50 μM GTP
11. 200 μM GTP

FIGURE 11C



1. No αβγ
2. No α
3. (standard)
4. +GTPγS
5. No L

FIGURE 12A

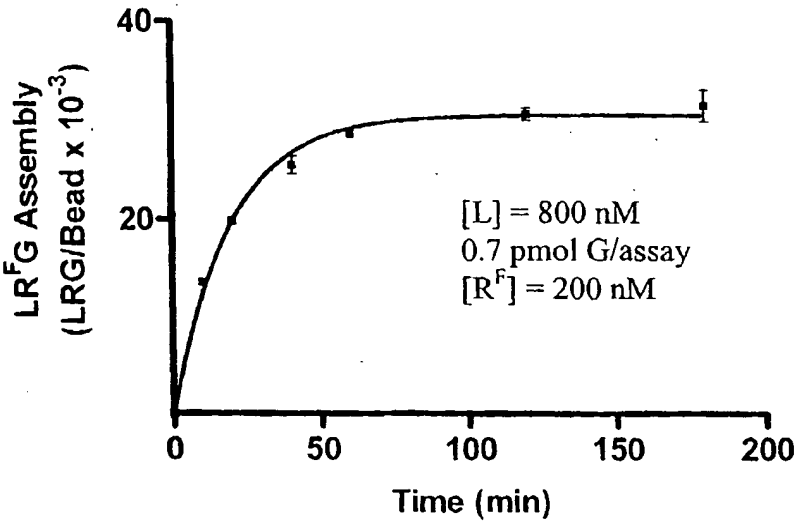


FIGURE 12B

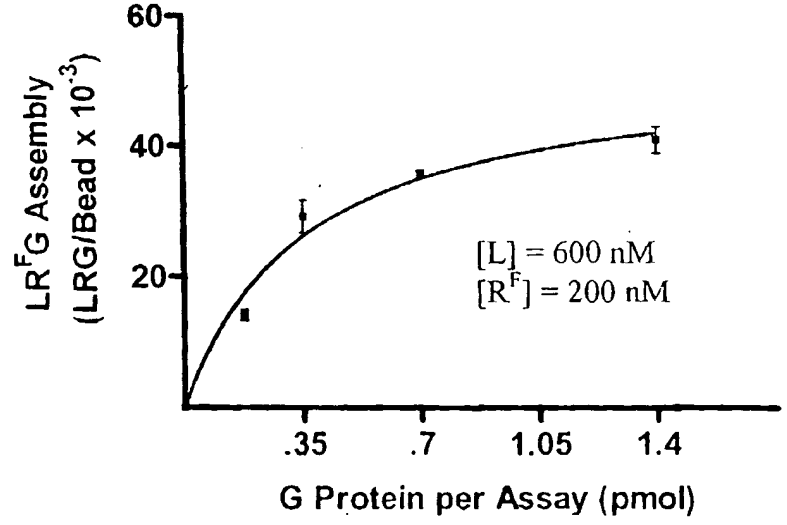


FIGURE 12C

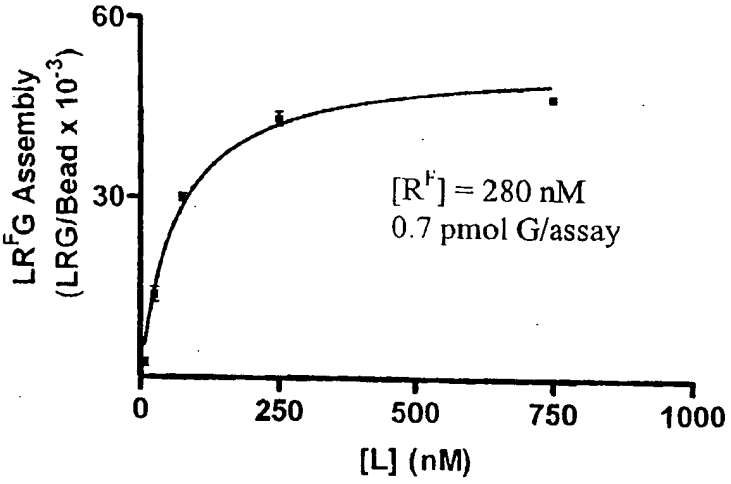


FIGURE 12D

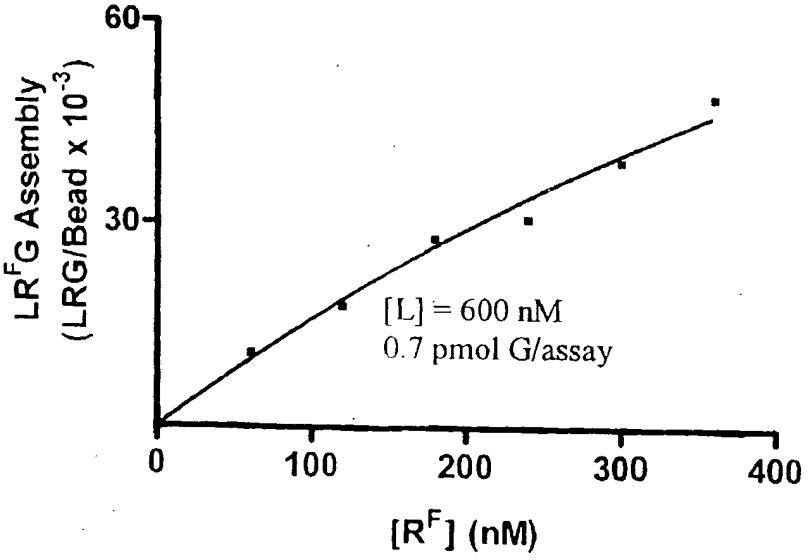


FIGURE 12E

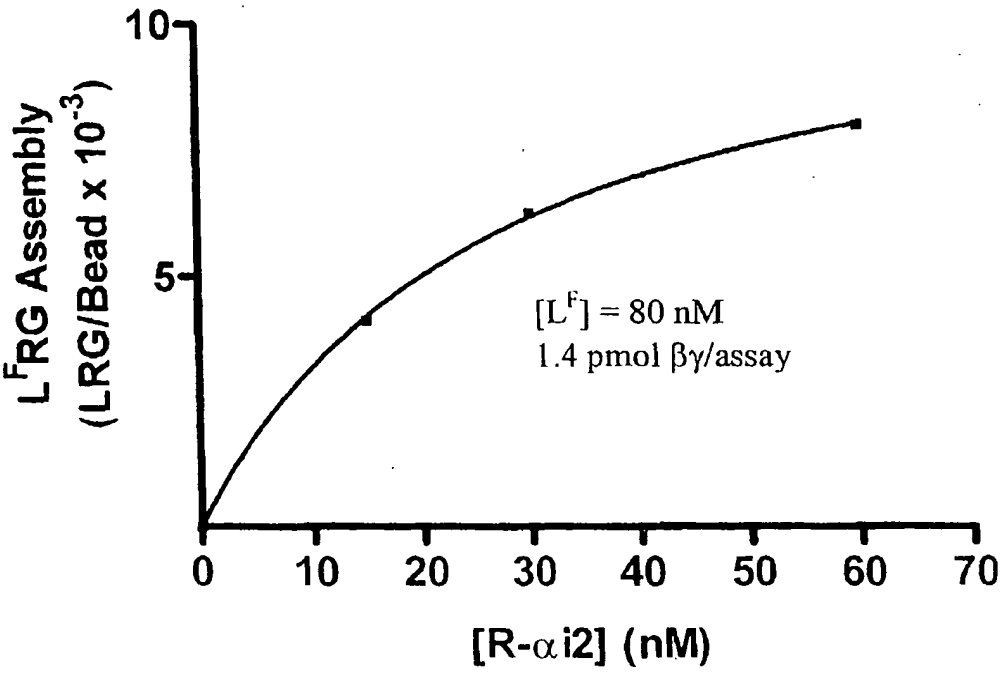

10
5
L^FRG Assembly
(LRG/Bead x 10^{-3})
$[L^F]$ = 80 nM
1.4 pmol βγ/assay
0
10
20
30
40
50
60
70
[R-αi2] (nM)

FIGURE 13A

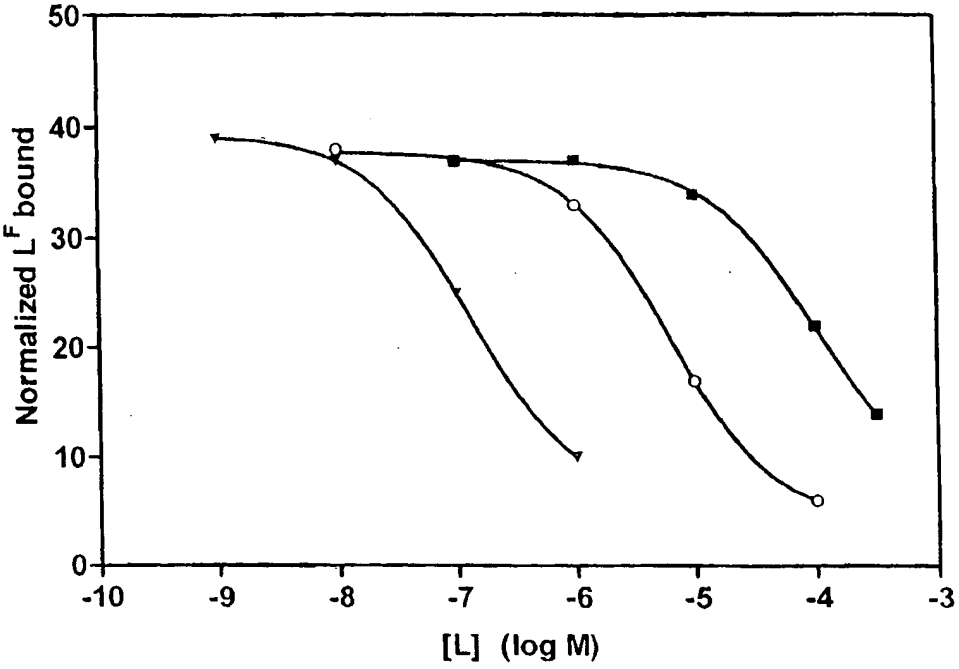


FIGURE 13B

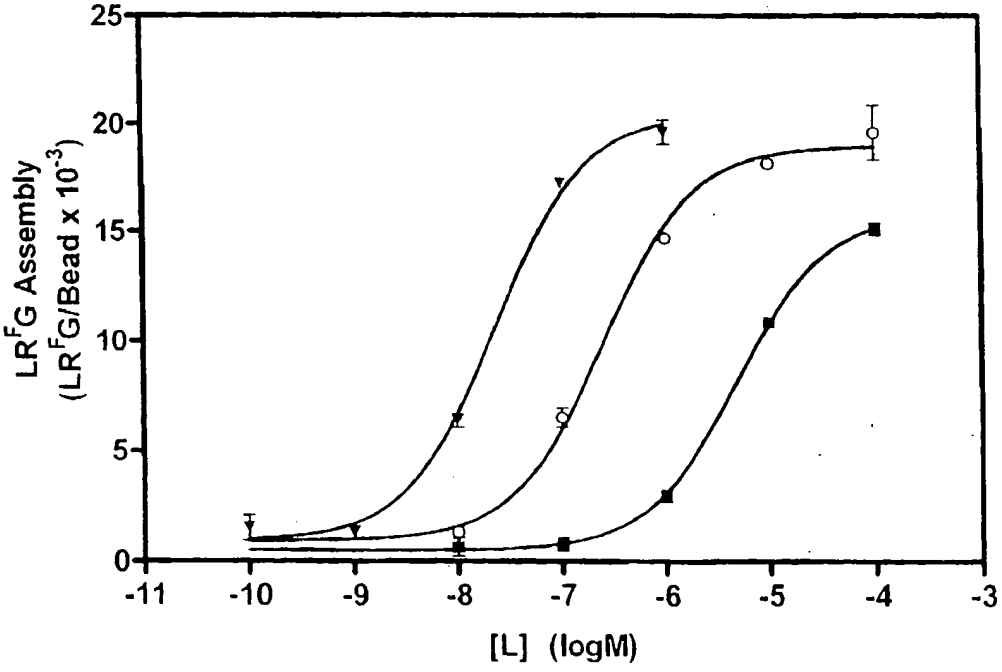


FIGURE 14

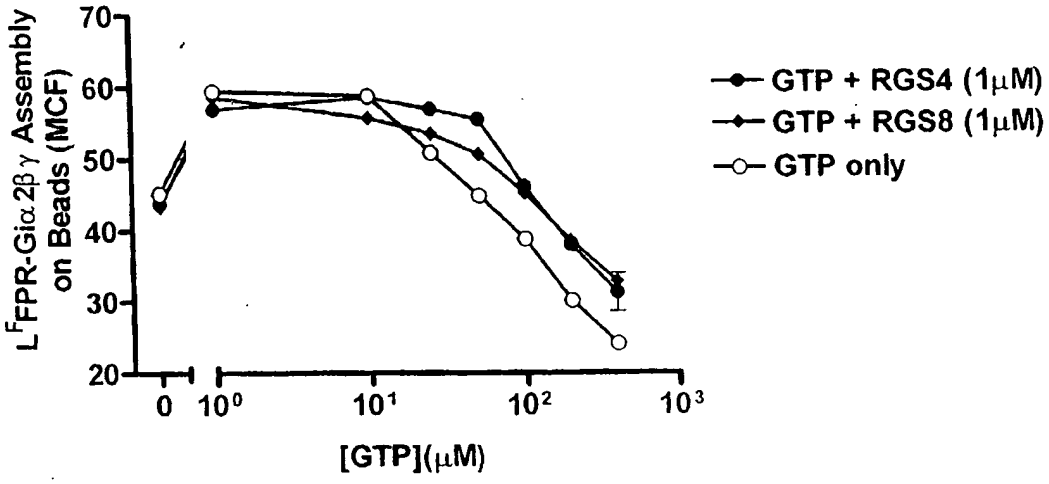

L^FFPR-Giα2βγ Assembly on Beads (MCF)
70
60
50
40
30
20
0
10^0
10^1
10^2
10^3
[GTP](μM)
GTP + RGS4 (1μM)
GTP + RGS8 (1μM)
GTP only

FIGURE 15A

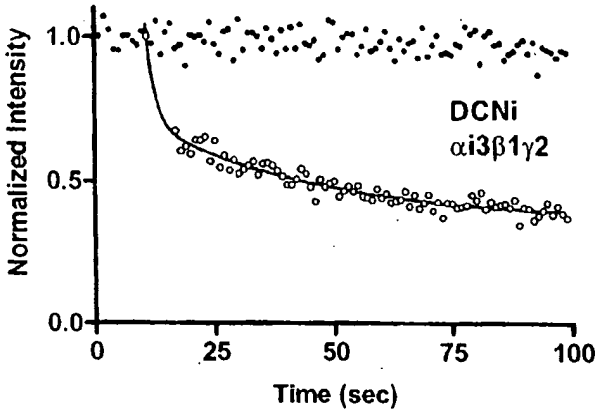


FIGURE 15B

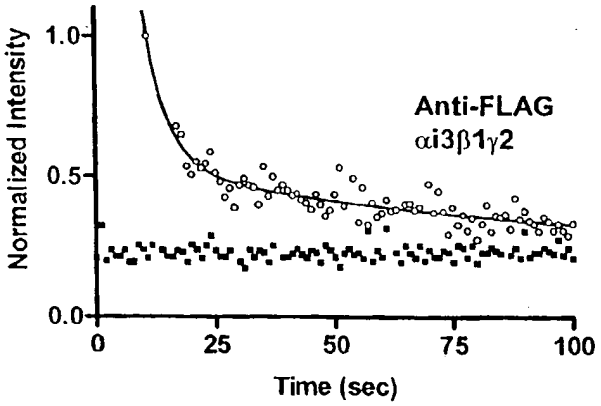


FIGURE 15C

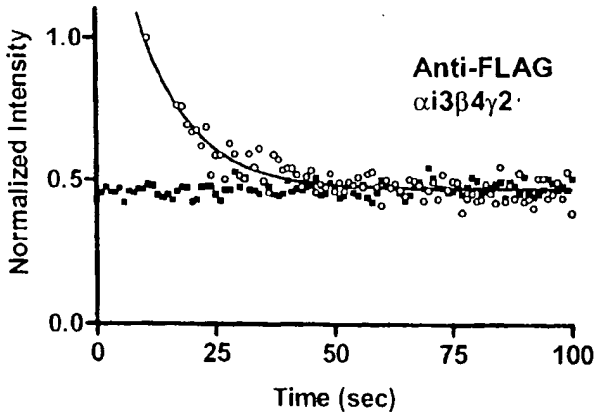


FIGURE 16

Lig-and	LR: K_d (nM)	ARG: EC_{50} (nM)	ARG Assembly
ALP	1.8 (0.1*)	NA	NA
ISO	220 (68)	180	100%
EPI	680 (370)	280	90%
NE	19,000 (10,000)	19,000	90%
SAL	2,300 (ND)	1,200	30%
DOB	2,400 (2,300)	2,600	10%